

The University of Chicago

THE PERMEABILITY OF THE LUNGS
TO ANTIBODIES

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PATHOLOGY

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THE PERMEABILITY OF THE LUNGS TO ANTIBODIES

JOHN P. FOX

From the Department of Pathology of the University of Chicago

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Although pulmonary permeability to many materials has been demonstrated, the permeability of the lungs to antibodies has not been satisfactorily determined. While recognizing the barrier formed by the lungs to particulate matter and bacteria, Besredka (1) nevertheless advocated the intratracheal injection of immune serum for the development of general passive immunity, especially where there existed some danger of anaphylactic shock. He claimed that a prompt immunity, lasting a week or more, and which would protect animals against from 30 to 50 times the lethal dose of diphtheria or tetanus toxin could be established. This assertion has been questioned by Jones (2, 3), who used both foreign and homologous agglutinating and hemolytic antisera, and concluded that the intratracheal route was a poor method of conferring general immunity. He found that intratracheal injection of such sera resulted in blood titers or hemolytic activity much inferior to those obtained after intraperitoneal injection of equal amounts of the same sera. Although there have been a few clinical attempts at local serum therapy, no other workers have actually experimented with the introduction of immune sera into the lungs. In view of this difference of opinion, the following experiments were performed in an attempt to gain further information concerning this question.

MATERIALS AND METHODS

Materials. Male rabbits weighing 2000 grams were used throughout except for one experiment utilizing 4 six-month-old puppies. Agglutinating sera were obtained from rabbits injected intravenously with formol-killed *B. typhosum* and *B. paratyphosum* B vaccines. Hemolytic serum was prepared from rabbits immunized with sheep cells. Lederle's

refined and concentrated type I anti-pneumococcus serum (Felton) was used for protective antibody. Laboratory strains of *B. typhosum* and *B. paratyphosum* B were used for vaccines. A culture of type I pneumococcus, isolated from a human case of lobar pneumonia, and virulent for mice up to 10^{-7} cc. of broth culture, was obtained from the laboratory of Dr. O. H. Robertson in the Department of Medicine. The inflammatory agent used consisted of 3 per cent starch and 2 per cent aleuronate, suspended in normal saline, and sterilized by boiling.

Methods of intratracheal injection. At first such injections into rabbits were made by means of a curved catheter, introduced through the mouth and larynx into the upper trachea of the unanesthetized animals, as described by Jones (4). To obtain deeper and more certain injection a similarly curved catheter was devised, containing a hard rubber ureteral catheter as a sliding core, which was tipped with a perforated olive to act as a rounded guide for the curved metal tube when the inner catheter was fully retracted. Once the metal catheter was in place, the inner core was pushed down at will into the bronchial tree. In later experiments, to insure intratracheal injection, the trachea was exposed through a small skin incision and injections were made directly through a needle. No anesthetics were necessary. In dogs the serum was introduced through a long atomizer inserted past the cocaineized larynx down to the bifurcation of the trachea, being sprayed slowly with each inspiration.

Collection of blood samples. Repeated blood samples were taken from the ear veins of rabbits. Dog blood samples were taken by vena puncture of the femoral veins.

Method of killing rabbits. The animals were put under heavy ether anesthesia and died while the thorax and abdomen were being opened. Tissues to be titrated were perfused by cannulation of the afferent vessels and cutting the veins. A solution of normal saline with 5 per cent sodium citrate was used to prevent clotting.

Extraction of tissues. Weighed samples of lung were ground in a mortar without sand, but with the addition of small amounts of saline. This was then diluted up to 1:15 on a volume-weight basis and allowed to remain in the icebox overnight or longer. In the early work this material was rediluted to 1:120 for centrifuging prior to titration, which was begun at this level. Later, as the need for working in lower dilutions became evident, the extracts were treated with staphylococcus vaccine to bring down non-specific flocculation and cleared by filtering through a Seitz or hard paper filter, thus allowing the titrations to

be started at the original dilution of 1:15. This is described in greater detail in another paper (5).

Titration. Serum titrations were begun at 1:15 or 1:30 dilutions. Tissue extract titrations were, as indicated above, begun at dilutions of 1:120 at first, but later at 1:15. Each successive dilution represented twice that of the preceding tube. Agglutinin titrations were carried out by adding from 3 to 5 drops of a suspension of living antigen (filtered through cotton) to each tube of serum or extract dilution. The tubes were incubated for 1 hour at 55°C. Readings were made with the aid of a hand lens after allowing the tubes to stand overnight in the icebox. Hemolysin titrations were made by the standard technique. Dilutions of serum or extract in 1 cc. volumes were inactivated at 55°C. for 30 minutes. To them were added 0.5 cc. of a 5 per cent saline suspension of freshly washed sheep cells and 0.2 cc. of complement (freshly prepared 1:4 dilution of guinea pig serum). Incubation was at 37°C. for 1 hour. Readings were made at once, and checked after allowing the tubes to stand overnight in the icebox.

Control observations included the titration of significant sera or tissue extracts for agglutinins against two closely related antigens, *B. typhosum* and *B. paratyphosum* B, the titration of normal rabbit tissues and sera, and other tissues from the experimental animals. Hemolysin controls consisted chiefly of titrating normal sera and extracts of normal lung tissues as well as the standard controls for the hemolytic system.

I. THE APPEARANCE OF ANTIBODIES IN THE BLOOD STREAM AFTER INTRATRACHEAL INJECTION OF ANTISERUM

Immune sera were introduced into the lungs of animals, and the extent of pulmonary permeability to antibodies determined by ascertaining the agglutinin or hemolysin titer of the blood, or by testing the degree of general passive immunity in protection against a virulent blood stream infection. Normal serum samples had been collected over a period of days before intratracheal administration of serum. Any later significant rise in serum titer or in resistance to blood stream infection indicated pulmonary permeability, the extent of which was determined by a comparison with serum titers or general resistance following the injection intravenously of equal amounts of immune sera into other animals, these results simulating those which would pertain

to a state of complete pulmonary permeability. Additional comparisons are afforded by the results of intragastric and subcutaneous injections of certain of the sera. While most of the work was on normal animals, in one series of experiments serum was introduced into inflamed lungs in order to determine the effect of inflammation upon pulmonary permeability.

A. The appearance of agglutinins in the blood stream

Throughout the following experiments, *B. typhosum* or *B. paratyphosum* B homologous antiserum was administered to rabbits in 4-cc. amounts. In order to check, roughly, lung permeability to antibodies contained in a foreign serum, similar rabbit agglutinating serum was used in 10-cc. doses in 4 young dogs. Experimental animals received agglutinating serum intratracheally, either by means of a catheter passed through the mouth, or directly into the exposed trachea by needle. Control animals received no serum or received it intravenously, subcutaneously or intragastrically. All animals were bled at intervals after injection of the immune serum, and the agglutinin content of such serum samples determined. See table 1.

Examination of the table shows the presence in normal serum of agglutinins for *B. paratyphosum* B, and that, due to natural causes or to variations in technic, there is a definite range of variability in the daily serum titers. Although not recorded in the table, similar agglutinins with a somewhat higher titer level were demonstrated for *B. typhosum*. In 7 rabbits out of the 15 injected intratracheally, i.e., nos. 44, 45, 47, 84, 85, 88, and 89, there is a definite rise in the titers of the post injection serum samples, more significant in the consistency maintained than in the degree of elevation. The peak of the rise tends to occur at from 48 to 72 hours after intratracheal injection. That these results were not due to alimentary permeability to antibody from inadvertently swallowed or misinjected serum is seen in the serum titers of rabbits 70 to 75 which were injected intragastrically, and which show no significant post injection rise. It may be assumed that antibody reached the lung in all the intratracheally injected animals, since in no case was the titer of the subsequently

TABLE 1

*Agglutinin titers of serum samples taken at intervals from normal unimmunized rabbits and from rabbits receiving agglutinating serum by various routes**

ROUTE OF INJECTION OF SERUM	RABBIT NUMBER	TITERS OF SERUM DRAWN AT INTERVALS								LUNG TITER AT DEATH	TITER OF SERUM INJECTED
		Before immunization	5 hours	12 hours	24 hours	48 hours	72 hours	96 hours	120 hours		
Intratracheal	28	15	15	15	30					120†	4,000
	29	60	60	60	60	60				120†	4,000
	32	15	15	15	15	15				960	4,000
	33	60	60	60	60	60	60	60		120†	4,000
	34	15†	15†	15†	15†	15†	15†	15†	15†	120†	4,000
	44	30	15	60	60					240	4,000
	45	15	15	15	30	30	30			120†	4,000
	46	15†	15	15†	15	15†	15†	15†		120†	4,000
	47	15†	15	30	60	60	120	120	60	120†	4,000
	84	30†			30	60	60			120	128,000
	85	30†			30	60	30			60	128,000
	86	30†			30†	30	30†	30†		240	128,000
	87	30†			30†	30†	30	30†		120	128,000
	88	30†			30	60	60	60	120	120	128,000
	89	30			30	60	60	60	60	60	128,000
Intravenous	30	15	240	240	120	120	120	120	120		4,000
	31	30	960	480	480	240	240	240	60		4,000
	76	240			480	480	480	480	480		128,000
	77	30†			960	480	480	480	480		128,000
	78	60			960	960	480	960	960		128,000
Subcutaneous	79	30			240	960	480	480	480		128,000
	80	30			480	960	480	480	480		128,000
	81	30†			240	1920	480	480	480		128,000
	82	30†			480	960	960	960	960		128,000
	83	30†			240	480	480	240	240		128,000
Intragastric	70	60			30	30	30†	30	Died		128,000
	71	30			30	30	30†	30†	30†		128,000
	72	30			30	30	30†	30†	30†		128,000
	73	30†			30	30	30	30†	30†		128,000
	74	60			30	30	30	30†	30		128,000
	75	30†			30	30†	30†	30†	30†		128,000
Normal rabbits vs. <i>B. Paratyphosum</i> B.	69a				60	60	30†	120	60		
	70a				30†	30	30†	30†	30†		
	71a				30†	30†	30†	30†	30		
	72a				30†	30†	30†	30†	30†		
	75a				30†	30†	30†	30†	30†		
	76a				30†	30†	30	30†	30		

* Serum injected in 4-cc. amounts. Figures in table indicate highest dilution in which agglutination detected. Thus 120 means 1:120. † is used to indicate that no agglutination was observed in dilution indicated, and which was the lowest dilution titrated.

made lung extract negative where dilutions below 1:120 were titrated, and a potent serum originally injected.

A comparison of the serum titers following intravenous administration of antiserum with those after intratracheal injection affords an indication of the degree of pulmonary permeability. The intravenously injected rabbits 30 and 31 may be compared with those intratracheally injected rabbits receiving antiserum of 1:4000 initial titer. Likewise, comparison may be made between rabbits 76-78 and 84-89, all of which received serum of 128,000 initial titer. The slight rise above the normal serum titers following intratracheal injection of immune serum, when com-

TABLE 2

*Agglutinin titers of serum samples taken at intervals from normal dogs before and after injection of rabbit agglutinating serum**

ROUTE OF INJECTION OF SERUM	DOG NUMBER	SERUM TITRATION								
		Days before injection				Days after injection				
		4	3	2	1	1	2	3	4	5
Subcutaneous	1	60	120	120	120	480	960	480	480	480
Intratracheal	7	60	30	30	60	120	120	60	60	60
	8	30	60	60	60	60	120	120	120	60
	9	60	60	60	60	60	120	120	60	60

* Titer of rabbit serum = 1:16,000. Serum injected in 10-cc. amounts. Symbols used the same as in table 1.

pared with that observed after intravenous injection, suggests that pulmonary permeability to antibody is similarly small in degree. A comparison between the titers of rabbits injected subcutaneously, and those of the intravenous group suggests that antibody introduced beneath the skin is somewhat delayed in its entrance into the blood stream, but that it eventually all arrives.

Table 2 presents the results obtained after injecting rabbit agglutinating serum into 4 dogs. The 3 dogs injected intratracheally show slight increases in subsequent serum titers of about the same magnitude as those found in the rabbit experiments. That this is an indication of permeability of about the same degree is suggested by a comparison with the titer levels obtained in the

one dog injected subcutaneously. We may conclude, therefore, that both rabbit and dog lungs are but slightly permeable to the agglutinins of immune rabbit sera.

B. The appearance of hemolysins in the blood stream

The possibilities of technical error in agglutinin determinations suggested the advisability of repeating these experiments with

TABLE 3

*Hemolysin titers of serum samples taken at intervals from normal rabbits before and after injection of hemolytic antiserum**

ROUTE OF INJECT- ING SERUM	RABBIT NUM- BER	SERUM TITER									LUNG TITER
		Days before injection				Days after injection					
		4	3	2	1	1	2	3	4	5	
Intratracheal	90	30	30	30	30	120	120				1920
	91	960	960	960	960	960	960	240			960
	92				240	240	120	240			480
	93	120	120	120	120	120	120	120	120		120
	94				240	240	240	240	120	120	15
	95		120	120	120	120	120				960
	96				240	240	480	480	480	480	240
	97				480	480	960	960	960		240
Intravenous	98				240	960	960	480	480	480	
	99				240	960	1920	960	480	240	
Subcutaneous	100				240	240	960	480	480	480	
	101				120	240	240	†			

* Titer of serum injected = 1:16,000. Serum given in 4-cc. amounts. The symbols used are the same as in table 1.

† Rabbit died.

other antibodies. Anti-sheep cell hemolytic serum in 4-cc. doses was, therefore, injected intratracheally, intravenously and subcutaneously into normal rabbits, and the resulting blood and lung titers determined.

The results (see table 3) may be briefly stated. Titrations of repeated serum samples from 4 normal rabbits show less variation than was noted in the normal agglutinin levels, but a surprisingly high normal average level of hemolytic activity. Of the

8 intratracheally injected rabbits, 3 showed a slight rise in the post injection titers. In comparison, the intravenous controls showed elevations much higher than those manifested in the intratracheal group. Of the 2 rabbits injected subcutaneously, one showed a delayed rise, equivalent in degree to that of the intravenous group, and the other died before a significant rise occurred.

The findings, thus, agree essentially with those observed with agglutinating sera. The rabbit lung is definitely, but only slightly permeable to the hemolytic antibodies contained in homologous serum.

C. The permeability of the lungs to protective antibodies

A final direct test of the normal lung permeability was conducted with protective antibodies contained in horse serum. In addition to affording a manifestation of still another type of antibody action, these experiments bear further evidence on the question of permeability to antibody contained in heterologous serum. Rabbits were given 4000 units of Type I anti-pneumococcus antibody solution intratracheally 24 hours prior to the intravenous injection of living virulent type I pneumococci. Control animals were given either the same amount of serum intravenously 24 hours before infection, or no serum at all. Resistance to the infection, as manifested by mortality and survival time, was taken as a measure of the amount of antibody circulating in the blood stream, the unprotected rabbits serving as a normal base line.

Table 4 presents the results. The infecting doses were varied somewhat in the hope that a better measure of the degree of protection would be observed, but, in view of the lack of correlation between the size of the infecting dose and the survival, it seems better merely to regard all the rabbits as being equivalently infected. The 3 normal rabbits died before 20 hours. The 6 protected intratracheally died between 28 and 70 hours. Of those protected intravenously, 2 survived, and the other 4 died between 6 and 8½ days. It seems evident then, on the basis of survival and mortality that the intratracheal group manifested a

slightly increased resistance to the intravenous infection over that shown by the normal rabbits, but very much less than that shown by the intravenous group. The degree of permeability to protective antibodies contained in heterologous serum, therefore, is of about the same magnitude as that indicated for agglutinins and hemolysins in homologous serum.

TABLE 4

*Survival of rabbits infected intravenously with type I pneumococci after receiving antiserum intratracheally or intravenously 24 hours earlier, or no serum at all**

ROUTE OF INJECTING SERUM	RABBIT NUMBER	INFECTING DOSE	SURVIVAL	POST MORTEM CULTURES	
				Blood	Spleen
Intratracheal	1a	$\frac{1}{4}$ slant	54-64 hours	Pn. I	Pn. I
	2a	$\frac{1}{4}$ slant	28 hours	Pn. I	Pn. I
	5a	$\frac{1}{2}$ slant	70 hours	Pn. I	Pn. I
	6a	$\frac{1}{2}$ slant	30-40 hours	Pn. I	Pn. I
	9a	$\frac{3}{4}$ slant	54-64 hours	Pn. I	Pn. I
	10a	$\frac{3}{4}$ slant	54-64 hours	Pn. I	Pn. I
Intravenous	3a	$\frac{1}{4}$ slant	6 days	Pn. I	Pn. I
	4a	$\frac{1}{4}$ slant	8 days	Pn. I	Pn. I
	7a	$\frac{1}{2}$ slant	8½ days	Pn. I	Pn. I
	8a	$\frac{1}{2}$ slant	Survived	***	***
	11a	$\frac{3}{4}$ slant	8 days	Pn. I	Pn. I
	12a	$\frac{3}{4}$ slant	Survived	***	***
No serum	13a	$\frac{1}{4}$ slant	Less than 20 hours	Pn. I	Pn. I
	14a	$\frac{1}{8}$ slant	Less than 20 hours	Pn. I	Pn. I
	15a	$\frac{1}{16}$ slant	Less than 20 hours	Pn. I	Pn. I

* Lederle's refined and concentrated anti-pneumococcus (type I) serum used in 4000-unit doses.

*** No cultures made. Rabbits survived.

D. The influence of inflammation on pulmonary permeability

In view of the possible use locally of immune serum in pulmonary disease, it is important to know the influence of pulmonary inflammation on lung permeability. Two brief series of experiments were undertaken to study the possibility that a significant alteration in permeability might occur. A mixture of starch and aleuronate in saline solution was used as an inflam-

matory agent, and was injected in 2-cc. doses by catheter into the lungs. In the first group of rabbits, this was mixed and injected with the immune serum to demonstrate the effect on permeability of a developing inflammation. In the second series of 6 rabbits, the starch-aleuronate preceded the serum by 48 hours so that the effects of an already established inflammation might be observed. The irritating effect of starch-aleuronate is well known, and the

TABLE 5

*Agglutinin titers of serum samples taken at intervals after intratracheal injection of agglutinating serum into rabbits with inflamed lungs**

STARCH-ALEURNOT INJECTED	RABBIT NUM- BER	SERUM TITER BE- FORE INJE- CTION	SERUM TITER AT INTERVALS AFTER INJECTION							LUNG TITRE	TITER OF SERUM IN- JECTED
			5 hours	12 hours	24 hours	48 hours	72 hours	96 hours	120 hours		
At time serum injected	52	15	15	15	15†	15†				120†	4,000
	53	15†	15†	15†	15†	15†	15			120†	4,000
	54	15	15	15	15	15	15	15		120†	4,000
	55	15	15	15	15	30	30	30	15	120†	4,000
	37	15†	15†	15†	15†	15†				480	4,000
	38	15†	15†	15	30	60	60	60		120†	4,000
	39	15†	15†	15†	15	60	60	30	60	120†	4,000
48 hours before serum in- jected	48	15	15	15	15					960	4,000
	49	15	15	15	15					960	4,000
	51	15	15†	15	15†					960	4,000
	59	120	120	120	120	120	120	120		480	32,000
	60	60	30	60	60	60	120	240		120	32,000
	61	60	60	60	120	120	120	120		480	32,000

* Serum injected in 4-cc. amounts. Three per cent starch and 2 per cent aleuronate injected in 2-cc. amounts. The symbols used are the same as in table 1.

resultant inflammation was verified both grossly and microscopically. The methods and materials, aside from the starch-aleuronate, were the same as in the previous agglutinin experiments, and the results are to be compared with those of that series.

It is seen (table 5) that a slight rise in serum agglutinin titer occurred in 4 out of 7 rabbits in which that starch-aleuronate and the serum were injected simultaneously and from which serum

samples were taken while the inflammation was developing. Of the group receiving serum 48 hours after the irritant, 2 out of 6 rabbits showed a slight similar rise in serum agglutinins.

The number of animals is too small to allow any conclusions differentiating between permeability in the presence of a developing inflammation and of an already established process. However, if we consider these two series as one group, we may certainly conclude that the presence of a starch-aleuronate inflammation in the lungs has no marked effect on pulmonary permeability to agglutinins.

II. THE PERSISTENCE OF ANTIBODY IN THE LUNGS AFTER INTRATRACHEAL INJECTION OF IMMUNE SERUM

It was felt that, by determination of the rate of disappearance from the lungs of intratracheally injected antibodies on the basis of titration of lung tissue extracts taken at various time intervals after injection, both an indirect measure of pulmonary permeability and an indication of the possible duration of a local passive immunity would be gained. Also, comparison with similar titrations made on the lungs of rabbits receiving serum intravenously would be of interest in the light of the relative antibody concentrations obtainable in the normal lungs by the two methods of serum administration.

All of the intratracheally injected rabbits were given serum in 4-cc. amounts, as were some of those intravenously injected, while the remainder of these received a maximal dose of 20 cc. The animals were killed at the time intervals indicated (see tables 6 and 7) up to 5 days after injection. Extracts were made of the perfused lung tissue and titrated, the dilutions representing a weight-volume ratio. Earlier experiments utilized a serum of low agglutinin titer, and the methods of titration limited the initial low dilution titratable to 1:120. These factors materially shortened the period of demonstrability of intratracheally injected antibodies. In the later experiments with agglutinins and in all the hemolysin work the serum was injected directly into the exposed trachea by needle, a much more potent serum was used, and methods of titration were employed permitting a low dilution of 1:15.

TABLE 6

*The antibody content of perfused normal rabbit lungs after intratracheal and intravenous injection of homologous agglutinating serum**

INTRATRACHEALLY INJECTED					INTRAVENOUSLY INJECTED					
Rabbit number	Titer of serum injected	Hours after injection	Lung titer	Serum titer at death	Rabbit number	Amount of serum given	Titer of serum injected	Hours after injections	Lung titer	Serum titer at death
						cc.				
1	64,000	1	560	70†	7	3	16,000	3	60†	240
2	64,000	1	560	70†	10	4	16,000	4	120†	480
3	64,000	2	1120	70†	11	4	16,000	4	120†	960
4	64,000	2	1120	70†	12	4	8,000	2	120	960
13	8,000	2	960	15	14	4	8,000	2	120	960
15	8,000	2	1920	15	16	4	8,000	4	120	960
5	16,000	3	960	15†	18	4	8,000	4	120†	960
6	16,000	3	240	15†	21	4	8,000	12	120	480
8	16,000	4	960	15†	23	4	8,000	12	120†	480
9	16,000	4	960	15†	25	4	8,000	24	120	480
17	8,000	4	1920	15†	27	4	8,000	24	120†	960
19	8,000	4	1920	15†	35	20	4,000	22	120†	960
20	8,000	12	960	15	36	20	4,000	22	120†	960
22	8,000	12	120	30	56	20	16,000	24	120†	480
24	8,000	24	960	30	57	20	16,000	$\frac{1}{2}$	120	960
26	8,000	24	1920	30	58	20	16,000	$\frac{1}{2}$	120	960
48	4,000	24	1920	30	62	20	32,000	20	60	3840
49	4,000	24	960	15	63	20	32,000	20	120	3840
51	4,000	24	960	15†	64	20	32,000	20	240	3840
28	4,000	48	120†	30	65	20	32,000	20	120	960
37	4,000	48	480	15†	66	20	32,000	20	60	3840
44	4,000	48	240	60	67	20	32,000	20	120†	3840
52	4,000	48	120†	60	68	20	32,000	20	240	1920
29	4,000	72	120†	15†	69	20	32,000	20	120	1920
32	4,000	72	960	15						
45	4,000	72	120†	30						
53	4,000	72	120†	15						
84	128,000	72	120	60						
85	128,000	72	60	60						
33	4,000	96	120†	60						
38	4,000	96	120†	60						
46	4,000	96	120†	15†						
54	4,000	96	120†	15						
59	32,000	96	480	120						
60	32,000	96	120	240						
61	32,000	96	480	120						
86	128,000	96	240	30						
87	128,000	96	120	30						
34	4,000	120	120†	15†						
39	4,000	120	120†	60						
47	4,000	120	120†	60						
55	4,000	120	120†	15						
88	128,000	120	120	60						
89	128,000	120	60	60						

* Serum injected intratracheally in 4-cc. amounts. The symbols used are the same as in table 1.

The results of the agglutinin experiments (see table 6) are based on 44 intratracheally and 24 intravenously injected rabbits. It is seen that definite antibody concentrations in the lung, as high as 1:1920, were easily demonstrated up to 24 hours after intratracheal injection. However, in no case, where dilutions below 1:120 were titrated, did the lung extract made during the 5 days after serum injection fail to exhibit agglutinins. The irregularity of titer level manifested in the lungs of rabbits injected with equally potent sera is probably explainable on the basis of uneven distribution of the injected sera in the lungs.

TABLE 7

*The hemolysin content of perfused normal rabbits lungs after intratracheal injection of homologous hemolytic serum**

	RAB- BIT 90	RAB- BIT 95	RAB- BIT 91	RAB- BIT 92	RAB- BIT 93	RAB- BIT 97	RAB- BIT 94	RAB- BIT 96	NORMAL RABBITS
	Hours after injection								
	48	48	72	72	96	96	120	120	
Serum titer.....	120	120	240	240	120	960	120	480	Ranges from 30 to 960
Lung titer.....	1920	960	960	480	120	240	15	240	All 15†

* Hemolytic serum was obtained from rabbits immunized with sheep cells, and was hemolytic in dilutions up to 1:36,000. Serum was injected in 4-cc. amounts. The symbols are the same as in table 1.

Coincidental titers of the blood sera of these intratracheal rabbits remained uniformly low, so that in most instances the lung titers surpass them, even in the late periods.

The lung titers of the rabbits injected intravenously present a striking contrast to those following intratracheal injection. Even when 20 cc., or five times the amount of serum given by way of the trachea, was injected, lung titers at 20 hours averaged lower than those in rabbits injected intratracheally, and sacrificed after 4 days. Attention is called particularly to rabbits 59 to 61 of the intratracheal group and to rabbits 62 to 69 of the intravenous group. The findings in the remaining intravenously injected animals, receiving less potent serum, and smaller doses are less significant but confirm at least the fact that the lungs do not tend

to accumulate marked amounts of antibody from the blood stream.

The results with the hemolysins (see table 7) represent a short confirmatory experiment confined to intratracheally injected rabbits killed in the later time periods only. A gradual diminution of lung titer is found extending over the periods from 48 to 120 hours, but even at the latter period appreciable amounts of hemolysin remain. While the accompanying blood serum titers at the 120-hour stage are somewhat higher than the lung titers, it should be recalled that the level of hemolytic activity in the normal serum is relatively high, while the titers of perfused lungs in a series of normal rabbits were always negative.

This prolonged retention of demonstrable amounts of antibody in the lung after intratracheal injection constitutes further evidence of the slight pulmonary permeability to antibodies. In addition, there is little evidence that antibodies in the circulating blood tend to accumulate in the normal lung, and one must conclude that local introduction is the best method of inducing a high antibody content in the normal lung.

DISCUSSION

These experiments lead to the conclusion that (a) normal lungs are only slightly permeable to antibodies and (b) that the presence of pulmonary inflammation induced with starch-aleuronate does not significantly alter this permeability. Furthermore, it appears that the degree of permeability is not significantly different (a), for antibodies contained in foreign or in homologous serum, or (b) for the three types of antibody tested.

These conclusions are essentially in accord with those reached by Jones (2), rather than with those of Besredka (1). The latter, it may be recalled, demonstrated the permeability of the lungs to antitoxins, and advocated the pulmonary route for general serum therapy. He failed, however, to compare his results with those to be obtained by administering equivalent amounts of antitoxin other than intratracheally. When such comparison is made, as done by Jones and by myself, it becomes apparent that the intratracheal route is a grossly inefficient way to induce a general passive immunity.

No attempt has been made in these experiments to study the mechanism of permeability. It is presumed that such permeability is essentially that of the tissues separating the blood and the airspaces, rather than representing lymphatic drainage which is confined to the peribronchial and perivascular tissues. According to Loosli (6), this separation is accomplished by the membrane of the capillary wall alone. Corper (7) has already suggested that pulmonary permeability is governed by laws similar to those governing the permeability of capillaries.

The established facts of pulmonary permeability further suggest this possibility. Studies with vital dyes (8), have shown the importance of such factors as molecular size, diffusibility, acidic or basic nature, and lipid solubility. It is known that gases, water, and most crystalloids pass readily from the lungs into the blood stream (7-11). The absorption of proteins has been largely studied by immunological and anaphylaxis experiments (11-16). Except for proteins of smallest molecular size, the lungs are but slightly protein permeable. For lipoids, viscous, and grossly particulate matter the alveolar phagocytes constitute the only mechanism of absorption (17-19). The degree of permeability manifested to antibodies, in the light of my experiments and those of Jones, most nearly approaches that exhibited for proteins in general.

It is perhaps surprising that inflammation should produce no apparent effect on permeability to antibody. Besredka (20), for instance, found that bile increased permeability to living paratyphoid bacteria. Furthermore, there is abundant evidence illustrating the changes that occur in capillary permeability in inflammation. It should be noted, however, these changes represent abnormal permeability in the direction away from the blood stream. Also, permeability to living motile bacteria is perhaps different from permeability to inert materials such as antibody. Finally, experimental support for my results may be found in the work of Corper (7), showing that acute inflammation actually decreased the pulmonary permeability to sodium sulphocyanate.

A final word may be said in regard to the clinical significance of

these experiments. In the first place, we have seen that the lungs do not afford a good route for inducing a general passive immunity. However, in pulmonary diseases amenable to serum therapy certain benefits accruing to local use of serum are suggested. In addition to the possible reduction of the peril of anaphylaxis as suggested by Besredka, one may enumerate particularly (1) the much higher local antibody content of the lung obtainable after local administration of small amounts of serum than follow injection of much larger amounts intravenously, and (2) the prolonged local retention of such antibody.

SUMMARY AND CONCLUSIONS

It has been shown that antibodies of three different types and contained in foreign or homologous serum appear in the blood stream but slowly and in slight degree after intratracheal injection. Conversely, such antibodies are retained in the lungs over a relatively prolonged period. These facts suggest that the lungs are only slightly permeable to antibody. Additional evidence indicated that the presence of sterile pulmonary inflammation exerted little effect on this permeability. On these grounds it was found impossible to agree with Besredka that the intratracheal route is a good way to induce general passive immunity.

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